Hypoglycemia-Associated Autonomic Failure in Advanced Type 2 Diabetes

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We tested the hypotheses that the glucagon response to hypoglycemia is reduced in patients who are approaching the insulin-deficient end of the spectrum of type 2 diabetes and that recent antecedent hypoglycemia shifts the glycemic thresholds for autonomic (including adrenomedullary epinephrine) and symptomatic responses to hypoglycemia to lower plasma glucose concentrations in type 2 diabetes. Hyperinsulinemic stepped hypoglycemic clamps (85, 75, 65, 55, and 45 mg/dl steps) were performed on two consecutive days, with an additional 2 h of hypoglycemia (50 mg/dl) in the afternoon of the first day, in 13 patients with type 2 diabetes—7 treated with oral hypoglycemic agents (OHA R_X; mean [±SD] HbA_{1c} 8.6 ± 1.1%) and 6 requiring therapy with insulin for an average of 5 years and with reduced C-peptide levels (insulin R_X, HbA_{1c} 7.5 ± 0.7%)—and 15 nondiabetic control subjects. The glucagon response to hypoglycemia was virtually absent (P = 0.0252) in the insulin-deficient type 2 diabetic patients (insulin R_X mean [±SE] final values of 52 ± 16 vs. 93 ± 15 pg/ml in control subjects and 98 ± 16 pg/ml in type 2 diabetic patients, OHA R_X on day 1). Glucagon (P = 0.0015), epinephrine (P = 0.0002), and norepinephrine (P = 0.0138) responses and neurogenic (P = 0.0149) and neuroglycopenic (P = 0.0015) symptom responses to hypoglycemia were reduced on day 2 after hypoglycemia on day 1 in type 2 diabetic patients; these responses were not eliminated, but their glycemic thresholds were shifted to lower plasma glucose concentrations. In addition, the glycemic thresholds for these responses were at higher-than-normal plasma glucose concentrations (P = 0.0082, 0.0028, 0.0023, and 0.0182, respectively) at baseline (day 1) in OHA R_X type 2 diabetic patients, with relatively poorly controlled diabetes. Because the glucagon response to falling plasma glucose levels is virtually absent and the glycemic thresholds for autonomic and symptomatic responses to hypoglycemia are shifted to lower glucose concentrations by recent antecedent hypoglycemia, patients with advanced type 2 diabetes, like those with type 1 diabetes, are at risk for hypoglycemia-associated autonomic failure and the resultant vicious cycle of recurrent iatrogenic hypoglycemia. *Diabetes* 51:724–733, 2002

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OHA, oral hypoglycemic agents.

In established (i.e., C-peptide negative) type 1 diabetes, plasma insulin levels do not decrease and plasma glucagon levels do not increase as plasma glucose levels fall (1–3). Two of the three important defenses against hypoglycemia are lost. The mechanism of the absent glucagon response is not known, but it is linked tightly to loss of endogenous insulin (4). Thus, patients with established type 1 diabetes are largely dependent on the remaining important defense against hypoglycemia, increased epinephrine secretion. However, largely as a result of recent antecedent iatrogenic hypoglycemia, the epinephrine response to falling plasma glucose levels is often attenuated in type 1 diabetes (1,3,5–7). In the setting of an absent glucagon response, an attenuated epinephrine response causes the clinical syndrome of defective glucose counterregulation (1). Affected patients are at 25-fold or greater risk of severe iatrogenic hypoglycemia than those with an intact epinephrine response (8,9). Reduced autonomic (including adrenomedullary epinephrine) and symptomatic responses also cause the clinical syndrome of hypoglycemia unawareness (1).

The concept of hypoglycemia-associated autonomic failure in type 1 diabetes (7,10,11) posits that recent antecedent iatrogenic hypoglycemia causes both defective glucose counterregulation (by reducing the epinephrine response in the setting of an absent glucagon response) and hypoglycemia unawareness (by reducing the autonomic and thus the neurogenic symptom response). Perhaps the most compelling support for this concept is the finding, in three independent laboratories, that hypoglycemia unawareness and at least in part the reduced epinephrine component of defective glucose counterregulation (but not the absent glucagon component) are reversible by as little as 2 weeks of scrupulous avoidance of iatrogenic hypoglycemia in most affected patients (12–14).

To what extent does the concept of hypoglycemia-associated autonomic failure apply to type 2 diabetes? The frequency of iatrogenic hypoglycemia is lower in type 2 diabetes than that in type 1 diabetes. Reported event rates for severe hypoglycemia (requiring the assistance of another individual) during aggressive insulin therapy in type 1 diabetes range from 62 (15) through 110 (16) to 170 (17) episodes per 100 patient-years. Those during aggressive insulin therapy in type 2 diabetes range from 3 (18) through 10 (19) (multiple daily injection data) to 73 (17) episodes per 100 patient-years. During 6 years of follow-up of patients with type 2 diabetes in the U.K. Prospective Diabetes Study, 2.4% of those using metformin, 3.3% of those using a sulfonylurea, and 11.2% of those using

TABLE 1
Mean (±SD) age, BMI, HbA_{1c}, years of OHA or insulin therapy, and fasting C-peptide concentrations, as well as gender distribution, in the nondiabetic subjects and patients with type 2 diabetes treated with OHA or insulin

	Nondiabetic	Type 2 diabetic patients	
		OHA Rx	Insulin Rx
Age (years)	50 ± 6	56 ± 6	57 ± 6
Female/male	8/7	4/3	3/3
BMI (kg/m^2)	30.2 ± 1.0	32.4 ± 2.7	34.0 ± 2.7
HbA_{1c} (%)	5.6 ± 0.4	8.6 ± 1.1	7.5 ± 0.7
Years of OHA or insulin Rx	—	10 ± 5	5 ± 3
Fasting C-peptide (ng/ml)*	2.3 ± 1.1	3.1 ± 1.7	1.1 ± 0.4†

*Multiply ng/ml by 0.331 to convert to nmol/l. †$P = 0.0051$ versus nondiabetic and 0.0172 versus type 2 diabetic OHA Rx.

insulin, reported major hypoglycemia (requiring medical attention or admission to a hospital) (20). Elsewhere (21), the U.K. Prospective Diabetes Study investigators noted that "patients often did not achieve normoglycemia. This was in part because of the high incidence of insulin-induced hypoglycemia, which is a limitation in treating patients with type 2 diabetes just as it is in treating patients with type 1 diabetes." Furthermore, in one series, the frequencies of severe hypoglycemia during the previous year were similar in type 2 diabetes (9 of 86 patients [10%]) and type 1 diabetes (14 of 86 patients [16%]) matched for duration of insulin therapy (22). Taken together, these data suggest that iatrogenic hypoglycemia becomes a progressively more frequent clinical problem for people with type 2 diabetes as they approach the insulin-deficient end of the spectrum of type 2 diabetes.

As noted earlier, an absent glucagon response to falling plasma glucose concentrations is a key pathophysiological feature of defective glucose counterregulation in type 1 diabetes. The glucagon response to hypoglycemia has been reported to be normal (23–25) or reduced but not absent (26–28) in type 2 diabetes. Given the evidence that the frequency of clinical hypoglycemia increases over time, just summarized, we tested the hypothesis that the glucagon response to hypoglycemia is reduced in patients who are approaching the insulin-deficient end of the spectrum of type 2 diabetes. As also noted earlier, an attenuated epinephrine response induced by previous hypoglycemia—in the setting of an absent glucagon response—causes defective glucose counterregulation, and an attenuated neurogenic symptom response induced by previous hypoglycemia causes hypoglycemia unawareness. Accordingly, we also tested the hypothesis that recent antecedent hypoglycemia shifts the glycemic thresholds for autonomic (including adrenomedullary epinephrine) and symptomatic responses to subsequent hypoglycemia to lower plasma glucose concentrations in type 2 diabetes.

RESEARCH DESIGN AND METHODS

Participants. A total of 13 patients with type 2 diabetes—7 treated with oral hypoglycemic agents (OHA R_X) and 6 treated with insulin—and 15 nondiabetic control subjects gave their written informed consent to participate in this study, which was approved by the Washington University Studies Committee and conducted at the Washington University General Clinical Research Center. The characteristics of the patients and the control subjects are listed in Table 1.

Exclusion criteria included the presence of circulating GAD antibodies, anemia, untreated hypertension, untreated proliferative retinopathy, renal insufficiency, autonomic neuropathy and clinically overt coronary, cerebral or peripheral macrovascular disease, and any additional contraindication to experimental hypoglycemia, such as known central nervous system disease including a history of seizures. Patients who were treated with OHA R_X were using a sulfonylurea, metformin or a thiazolidinedione, or a combination of these. Patients who were treated with insulin were selected for a relatively long-standing requirement for therapy with insulin. The intent was not to contrast impacts of the types of therapy but rather to use the requirement for long-term insulin therapy as an index of advanced type 2 diabetes. The effectiveness of that selection process is supported by the lower fasting plasma C-peptide concentrations in the patients who were treated with insulin ($P = 0.0051$ vs. nondiabetic subjects and 0.0172 vs. OHA R_X type 2 diabetic patients, Table 1).

Experimental design. The participants were studied, after overnight fasts, on two consecutive days. Hyperinsulinemic (2.0 $mU \cdot kg^{-1} \cdot min^{-1}$, 12.0 $pmol \cdot kg^{-1} \cdot min^{-1}$) stepped hypoglycemic clamps (29) (85, 75, 65, 55 and 45 mg/dl, 4.7, 4.2, 3.6, 3.3, and 2.5 mmol/l, in hourly steps) were performed on the morning of day 1 and of day 2 with an additional 2-h period of hypoglycemia (50 mg/dl, 2.8 mmol/l) on the afternoon of day 1. Arterialized venous samples, with the participants in the supine position throughout, were drawn from a line inserted in a hand vein (with that hand kept in an ~60°C plexiglas box) at −30, −15, and 0 min and at 30-min intervals through 300 min. Insulin and glucose (20%) were infused through a line in an antecubital vein with the glucose infusion rate varied, based on plasma glucose measurements every 5 min, to clamp glucose levels at the target levels. Blood pressure and heart rate were also recorded (Propaq, Encore, Protocol Systems, Beverton, OR) at 30-min intervals; the electrocardiogram was monitored throughout.

Analytical methods. Plasma glucose was measured with a glucose oxidase method (Glucose Analyzer 2; Beckman, Brea, CA). Plasma insulin (30), C-peptide (30), and glucagon (31) were measured with radioimmunoassays, plasma epinephrine, and norepinephrine with a single isotope derivative (radioenzymatic) method (32). Symptoms of hypoglycemia were quantified by asking the participants to score (0, none to 6, severe) each of 12 symptoms: 6 neurogenic symptoms (adrenergic: heart pounding, shaky/tremulous, and nervous/anxious; cholinergic: sweaty, hungry, and tingling) and 6 neuroglycopenic symptoms (difficulty thinking/confused, tired/drowsy, weak, warm, faint and dizzy) based on our published data (33).

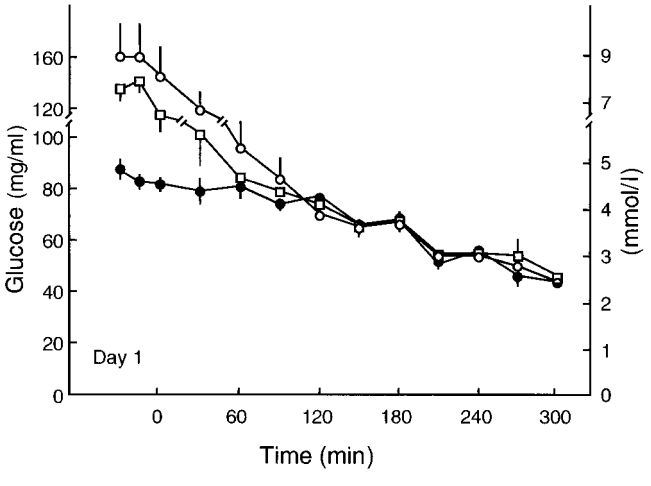

FIG. 1. Mean (±SE) plasma glucose concentrations during hyperinsulinemic stepped hypoglycemic clamps on the morning of day 1 in nondiabetic subjects (●) ($n = 15$) and in patients with type 2 diabetes treated with OHA (○) ($n = 7$) and treated with insulin (□) ($n = 6$).

TABLE 2
Mean (±SE) plasma glucose concentrations (mg/dl*) during hyperinsulinemic stepped hypoglycemic clamps on the morning and, after an additional 2 h of hypoglycemia (50 mg/dl), the afternoon of day 1 and on the morning of day 2 in nondiabetic subjects (n = 15) and patients with type 2 diabetes treated with OHA (n = 7) or with insulin (n = 6)

	Time (min)												
	−30	−15	0	30	60	90	120	150	180	210	240	270	300
Nondiabetic subjects													
Day 1	88 ± 4	88 ± 3	82 ± 2	79 ± 5	81 ± 2	74 ± 2	77 ± 1	66 ± 2	68 ± 3	52 ± 3	56 ± 1	46 ± 2	44 ± 2
Day 2	86 ± 3	89 ± 1	88 ± 3	80 ± 3	79 ± 5	75 ± 2	77 ± 2	63 ± 2	64 ± 2	55 ± 2	53 ± 2	46 ± 1	42 ± 2
Type 2 diabetic patients													
OHA Rx													
Day 1	161 ± 25	161 ± 25	146 ± 22	120 ± 13	96 ± 10	84 ± 4	70 ± 3	65 ± 2	67 ± 4	54 ± 2	54 ± 2	50 ± 2	44 ± 12
Day 2	92 ± 4	92 ± 3	88 ± 3	88 ± 1	83 ± 4	74 ± 2	74 ± 4	62 ± 2	64 ± 1	53 ± 3	53 ± 2	45 ± 3	45 ± 1
Insulin Rx													
Day 1	136 ± 10	142 ± 10	116 ± 13	101 ± 12	84 ± 5	79 ± 4	74 ± 4	66 ± 5	68 ± 3	55 ± 2	55 ± 3	54 ± 5	46 ± 3
Day 2	108 ± 12	103 ± 11	106 ± 12	103 ± 12	97 ± 6	77 ± 4	74 ± 4	68 ± 3	67 ± 5	61 ± 4	54 ± 3	50 ± 2	47 ± 2

*Multiply mg/dl by 0.0551 to convert to mmol/l.

TABLE 3
Mean (±SE) plasma insulin concentrations (μU/ml*) during hyperinsulinemic stepped hypoglycemic clamps on the morning and, after an additional 2 h of hypoglycemia (50 mg/dl), the afternoon of day 1 and on the morning of day 2 in nondiabetic subjects (n = 15) and patients with type 2 diabetes treated with OHA (n = 7) or insulin (n = 6)

	Time (min)													P
	−120	−30	0	30	60	90	120	150	180	210	240	270	300	
Nondiabetic subjects														
Day 1	10 ± 2	53 ± 3	56 ± 6	96 ± 11	117 ± 12	106 ± 13	120 ± 12	123 ± 12	126 ± 8	127 ± 9	121 ± 12	137 ± 8	141 ± 8	
Day 2	6 ± 4	56 ± 4	60 ± 5	108 ± 7	113 ± 6	121 ± 7	120 ± 6	123 ± 9	116 ± 11	130 ± 10	120 ± 10	118 ± 8	128 ± 7	0.1920
Type 2 diabetic patients														
OHA Rx†														
Day 1	16 ± 4	95 ± 21	138 ± 32	210 ± 51	264 ± 61	248 ± 78	267 ± 83	239 ± 50	222 ± 54	210 ± 47	204 ± 37	203 ± 60	215 ± 31	
Day 2	40 ± 13	57 ± 8	96 ± 23	110 ± 22	114 ± 29	138 ± 25	145 ± 26	154 ± 26	162 ± 27	148 ± 29	159 ± 27	173 ± 27	227 ± 51	0.4441
Insulin Rx‡														
Day 1	17 ± 5	53 ± 14	95 ± 22	264 ± 27	344 ± 52	311 ± 54	310 ± 50	302 ± 45	292 ± 44	302 ± 46	288 ± 42	313 ± 49	296 ± 68	
Day 2	24 ± 4	41 ± 12	51 ± 11	211 ± 32	270 ± 40	288 ± 46	305 ± 45	306 ± 54	307 ± 50	300 ± 45	299 ± 48	329 ± 52	317 ± 52	0.9253

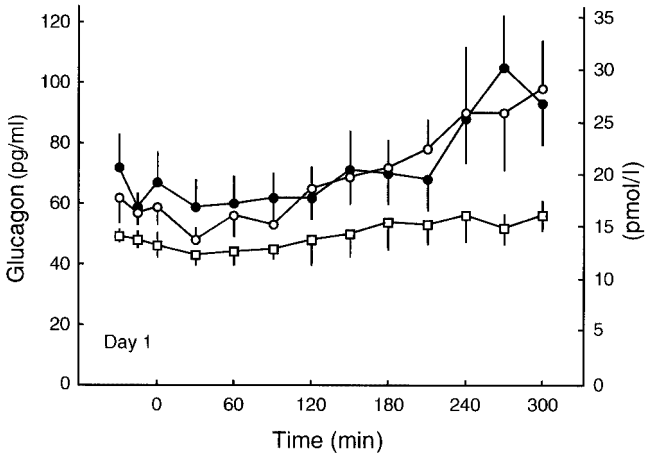

FIG. 2. Mean (±SE) plasma glucagon concentrations during hyperinsulinemic stepped hypoglycemic clamps on the morning of day 1 in nondiabetic subjects (●) (n = 15) and in patients with type 2 diabetes treated with OHA (○) (n = 7) and treated with insulin (□) (n = 6). P = 0.0252 for nondiabetic versus type 2 diabetic insulin R_x contrast.

Statistical methods. Data are expressed as the mean ± SE except where the SD is specified. Data were analyzed by general linear model repeated measures ANOVA. $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Glucose clamps. Target plasma glucose concentrations were approximated during the hyperinsulinemic stepped hypoglycemic clamps in all three groups of participants on days 1 and 2 (Fig. 1, Table 2). Plasma insulin concentrations were higher in both groups of patients with type 2 diabetes than in the nondiabetic subjects, but there were no significant differences between the day 1 and day 2 insulin levels during the hyperinsulinemic clamps in any of the three groups of participants (Table 3).

Responses to hypoglycemia on day 1. Compared with that of nondiabetic control subjects, the plasma glucagon response to hypoglycemia was reduced (P = 0.0252) in insulin R_x type 2 diabetic patients but not in OHA R_x type 2 diabetic patients (Fig. 2, Table 4). Indeed, the glucagon response was virtually absent in the type 2 diabetes insulin R_X group.

The plasma epinephrine response to hypoglycemia was increased in the type 2 diabetes OHA R_X group (P = 0.0082 versus nondiabetic), i.e., it occurred at higher plasma glucose concentrations (Fig. 3, Table 5). Although the epinephrine response seemed to be reduced at the lowest plasma glucose concentration in the type 2 diabetes insulin R_X group (Fig. 3), that seeming difference was not statistically significant. The plasma norepinephrine response to hypoglycemia was also increased in the type 2 diabetes OHA R_X group (P = 0.0082 versus nondiabetic; Fig. 4, Table 6).

The neurogenic (autonomic) symptom response to hypoglycemia was increased in the type 2 diabetes OHA R_X group (P = 0.0023 versus nondiabetic; Fig. 5, Table 7). The neuroglycopenic symptom response to hypoglycemia was also increased in the type 2 diabetes OHA R_X group (P = 0.0182 versus nondiabetic; Table 8).

TABLE 4
Mean (±SE) plasma glucagon concentrations (pg/ml*) during hyperinsulinemic stepped hypoglycemic clamps on the morning and, after an additional 2 h of hypoglycemia (50 mg/dl), the afternoon of day 1 and on the morning of day 2 in nondiabetic subjects (n = 15) and patients with type 2 diabetes treated with OHA (n = 7) or insulin (n = 6)

	Time (min)												
	−30	−15	0	30	60	90	120	150	180	210	240	270	300	P
Nondiabetic subjects														
Day 1	72 ± 12	59 ± 5	67 ± 11	59 ± 10	60 ± 10	62 ± 9	62 ± 11	71 ± 14	70 ± 2	68 ± 10	88 ± 15	105 ± 18	93 ± 15	
Day 2	54 ± 6	55 ± 6	50 ± 5	49 ± 5	47 ± 5	48 ± 5	45 ± 5	51 ± 4	46 ± 6	46 ± 6	63 ± 43	69 ± 12	73 ± 10	0.0249
Type 2 diabetic subjects														
OHA Rx†														
Day 1	62 ± 8	57 ± 4	59 ± 6	48 ± 4	56 ± 7	53 ± 7	65 ± 11	69 ± 10	72 ± 13	78 ± 11	90 ± 22	90 ± 22	98 ± 16	
Day 2	47 ± 7	55 ± 9	48 ± 6	42 ± 6	39 ± 5	39 ± 9	41 ± 8	39 ± 5	48 ± 13	42 ± 6	55 ± 7	62 ± 9	52 ± 16	0.0036
Insulin Rx†‡														
Day 1	49 ± 2	48 ± 3	46 ± 4	43 ± 4	44 ± 5	45 ± 4	48 ± 9	50 ± 8	54 ± 10	53 ± 7	56 ± 9	52 ± 5	56 ± 5	
Day 2	44 ± 6	45 ± 3	48 ± 4	45 ± 5	37 ± 3	38 ± 5	39 ± 5	34 ± 3	33 ± 4	42 ± 9	45 ± 8	48 ± 9	51 ± 8	0.0821

*Multiply pg/dl by 0.02871 to convert to pmol/l; †type 2 diabetic (n = 13) P = 0.0015 day 2 versus day 1; ‡day 1 P = 0.0252 versus nondiabetic.

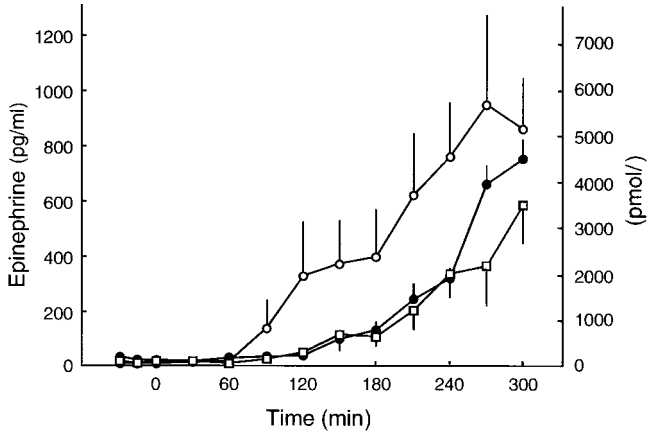

FIG. 3. Mean (±SE) plasma epinephrine concentrations during hyperinsulinemic stepped hypoglycemic clamps on the morning of day 1 in nondiabetic subjects (●) (n = 15) and in patients with type 2 diabetes treated with OHA (○) (n = 7) and treated with insulin (□) (n = 6). P = 0.0082 for nondiabetic versus type 2 diabetic OHA R_X contrast.

Impact of two episodes of hypoglycemia on day 1 on the responses to hypoglycemia on day 2. Hypoglycemia on day 1 was associated with reduced plasma glucagon (P = 0.0249; Table 4), plasma epinephrine (P = 0.0001; Table 5), plasma norepinephrine (P = 0.0106; Table 6), and neuroglycopenic symptom (P = 0.0467; Table 8) responses to hypoglycemia on day 2 in nondiabetic subjects. The neurogenic symptom response tended to be reduced (P = 0.1488; Table 7).

In the patients with type 2 diabetes as a group (n = 13), hypoglycemia on day 1 was associated with reduced plasma glucagon (P = 0.0015), plasma epinephrine (P = 0.0002), plasma norepinephrine (P = 0.0138), neurogenic symptom (P = 0.0149), and neuroglycopenic symptom (P = 0.0015) responses to hypoglycemia on day 2 (group data not shown).

Hypoglycemia on day 1 was associated with reduced plasma glucagon (P = 0.0036; Fig. 6, Table 4), plasma epinephrine (P = 0.0022; Fig. 7, Table 5), plasma norepinephrine (P = 0.0441; Fig. 8, Table 6), neurogenic symptom (P = 0.0306; Fig. 9, Table 7), and neuroglycopenic symptom (P = 0.0097; Table 8) responses on day 2 in the type 2 diabetes OHA R_X group.

Hypoglycemia on day 1 was associated with apparently but not significantly reduced plasma glucagon (P = 0.0821; Table 4), plasma epinephrine (P = 0.0740; Table 5), and plasma norepinephrine (P = 0.1434; Table 6) responses to hypoglycemia on day 2 in the type 2 diabetes insulin R_X group. Neurogenic symptom (P = 0.0202; Table 7) and neuroglycopenic symptom (P = 0.0082; Table 8) responses to hypoglycemia were reduced significantly.

There were no significant differences in heart rate or in systolic or diastolic blood pressure among the three groups (data not shown).

DISCUSSION

These data support our hypotheses that the glucagon response to hypoglycemia is reduced in patients who are approaching the insulin-deficient end of the spectrum of type 2 diabetes and that recent antecedent hypoglycemia shifts glycemic thresholds for autonomic (including adrenomedullary epinephrine) and symptomatic responses to

TABLE 5
Mean (±SE) plasma epinephrine concentrations (pg/ml*) during hyperinsulinemic stepped hypoglycemic clamps on the morning and, after an additional 2 h of hypoglycemia (50 mg/dl), the afternoon of day 1 and on the morning of day 2 in nondiabetic subjects (n = 15) and patients with type 2 diabetes treated with OHA (n = 7) or insulin (n = 6)

	Time (min)												
	−30	−15	0	30	60	90	120	150	180	210	240	270	300	P
Nondiabetic subjects														
Day 1	40 ± 17	30 ± 7	30 ± 7	24 ± 5	38 ± 13	42 ± 10	45 ± 10	111 ± 38	147 ± 35	270 ± 64	353 ± 42	728 ± 74	830 ± 79	
Day 2	31 ± 7	37 ± 9	35 ± 9	37 ± 9	37 ± 7	34 ± 8	40 ± 8	51 ± 13	69 ± 21	110 ± 28	227 ± 73	368 ± 70	587 ± 83	0.0001
Type 2 diabetic patients														
OHA Rx†‡														
Day 1	17 ± 2	14 ± 2	15 ± 3	18 ± 4	26 ± 5	156 ± 11	362 ± 218	410 ± 173	437 ± 188	686 ± 246	836 ± 266	1043 ± 356	946 ± 203	
Day 2	31 ± 5	23 ± 5	23 ± 5	29 ± 5	29 ± 4	37 ± 9	62 ± 21	76 ± 29	76 ± 30	159 ± 81	156 ± 44	322 ± 84	439 ± 128	0.0022
Insulin Rx†														
Day 1	22 ± 6	14 ± 4	24 ± 9	26 ± 9	17 ± 4	35 ± 16	600 ± 20	130 ± 65	121 ± 41	225 ± 78	372 ± 98	403 ± 98	403 ± 158	
Day 2	19 ± 3	17 ± 2	20 ± 5	18 ± 3	20 ± 4	25 ± 5	24 ± 8	25 ± 8	35 ± 11	58 ± 28	145 ± 69	195 ± 88	352 ± 151	0.0740

*Multiply pg/ml by 5.458 to convert to pmol/l; †type 2 diabetic (n = 13) P = 0.0002 day 2 versus day 1; ‡day 1 P = 0.0082 versus nondiabetic.

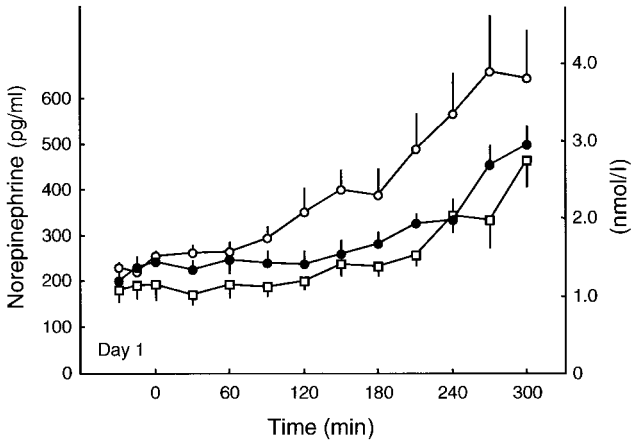

FIG. 4. Mean (±SE) plasma norepinephrine concentrations during hyperinsulinemic stepped hypoglycemic clamps on the morning of day 1 in nondiabetic subjects (●) (n = 15) and in patients with type 2 diabetes treated with OHA (○) (n = 7) and treated with insulin (□) (n = 6). P = 0.0082 for nondiabetic versus type 2 diabetic OHA R_X contrast.

subsequent hypoglycemia to lower plasma glucose concentrations in type 2 diabetes. They also indicate that the glycemic thresholds for these responses are shifted to higher plasma glucose concentrations at baseline (day 1) in patients with poorly controlled type 2 diabetes.

An absent glucagon response to falling plasma glucose concentrations (2–4) is a key pathophysiological feature of the clinical syndrome of defective glucose counterregulation (1,8,9) and the concept of hypoglycemia-associated autonomic failure in type 1 diabetes (1,7,10,11). Because the absent glucagon response is linked tightly with the loss of endogenous insulin secretion in type 1 diabetes (4), we reasoned that the glucagon response might be reduced in patients who are approaching the insulin-deficient end of the spectrum of type 2 diabetes. Accordingly, we selected patients who required insulin therapy for management of their type 2 diabetes for an average of 5 years, a clinical surrogate for insulin deficiency that was supported by the finding of significantly reduced plasma C-peptide levels. Indeed, the mean fasting plasma C-peptide concentration of 1.1 ng/ml in these patients was lower than the optimal cutoff value of 1.3 ng/ml or less found by Berger et al. (34) to distinguish type 1 diabetes from typical type 2 diabetes. We also required the absence of circulating GAD antibodies to exclude patients with late-onset type 1 diabetes (35,36). Thus, our selection process effectively identified patients with insulin-deficient type 2 diabetes. In these patients with advanced type 2 diabetes, the glucagon response to hypoglycemia was virtually absent, just as it is in type 1 diabetes (2–4). This is a statistically robust finding because it was documented with a rather small sample of appropriately identified patients whose glucagon responses were compared with those of age-, gender-, and BMI-matched nondiabetic control subjects.

Plasma insulin concentrations were higher at baseline and during the hyperinsulinemic stepped hypoglycemic clamps in both groups of patients with type 2 diabetes, although there were no significant differences between day 1 and day 2 insulin levels in any of the three groups. These supraphysiological insulin levels permitted accurate matching of plasma glucose concentrations at euglycemic

TABLE 6
Mean (± SE) plasma norepinephrine concentrations (pg/ml*) during hyperinsulinemic stepped hypoglycemic clamps on the morning and, after an dditional 2 h of hypoglycemia (50 mg/dl), the afternoon of day 1 and on the morning of day 2 in nondiabetic subjects (n = 15) and patients with type 2 diabetes treated with OHA (n = 7) or insulin (n = 6)

	Time (min)											
	−30	−15	0	30	60	90	120	150	180	210	240	270	300	P
Nondiabetic subjects														
Day 1	202 ± 22	231 ± 25	242 ± 25	227 ± 22	248 ± 29	241 ± 28	238 ± 29	260 ± 33	282 ± 27	326 ± 24	334 ± 24	456 ± 43	501 ± 40	
Day 2	213 ± 26	220 ± 25	250 ± 31	237 ± 29	249 ± 26	254 ± 23	237 ± 26	244 ± 26	245 ± 27	240 ± 25	316 ± 37	343 ± 22	448 ± 22	0.0106
Type 2 diabetic patients														
OHA Rx†‡														
Day 1	232 ± 11	222 ± 9	255 ± 12	263 ± 17	265 ± 22	296 ± 25	352 ± 53	402 ± 43	389 ± 59	491 ± 79	567 ± 90	660 ± 123	646 ± 106	
Day 2	240 ± 23	244 ± 29	247 ± 30	250 ± 23	260 ± 14	259 ± 13	289 ± 22	288 ± 26	296 ± 25	335 ± 80	358 ± 60	484 ± 104	602 ± 125	0.0441
Insulin Rx†														
Day 1	182 ± 28	191 ± 30	193 ± 33	171 ± 22	193 ± 29	188 ± 22	202 ± 21	239 ± 27	234 ± 23	257 ± 23	345 ± 37	335 ± 61	465 ± 60	
Day 2	192 ± 38	175 ± 28	171 ± 32	193 ± 47	182 ± 32	197 ± 54	193 ± 50	201 ± 48	205 ± 27	214 ± 50	238 ± 66	258 ± 58	339 ± 81	0.1434

*Multiply pg/ml by 0.005911 to convert to nmol/l; †type 2 diabetic (n = 13) P = 0.0138 day 2 versus day 1; ‡day 1 P = 0.0028 versus nondiabetic.

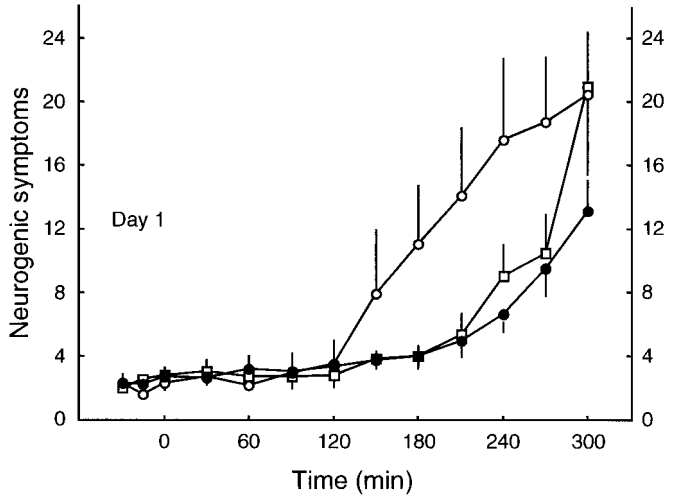

FIG. 5. Mean (±SE) neurogenic (autonomic) symptom scores during hyperinsulinemic stepped hypoglycemic clamps on the morning of day 1 in nondiabetic subjects (●) (n = 15) and in patients with type 2 diabetes treated with OHA (○) (n = 7) and treated with insulin (□) (n = 6). P = 0.0023 for nondiabetic versus type 2 diabetic OHA R_x contrast.

and progressively hypoglycemic (to 45 mg/dl) levels. Such matching of the stimulus is critical to the interpretation of the neuroendocrine and symptomatic responses to hypoglycemia. However, hyperinsulinemia per se cannot explain the reduced glucagon response to hypoglycemia in the insulin-deficient (type 2 diabetic insulin R_x) patients because there was a normal glucagon response to hypoglycemia in the insulin-sufficient (type 2 diabetes OHA R_x) patients despite comparable hyperinsulinemia. We also considered the possibility that unrecognized recent antecedent iatrogenic hypoglycemia might have caused the reduced glucagon response to hypoglycemia in the (insulin-treated) insulin-deficient patients. Because the patients were studied as General Clinical Research Center inpatients, with frequent plasma glucose measurements overnight before the glucose clamps, we know that hypoglycemia did not occur the night before the study. We cannot be absolutely certain that it did not occur before then. However, the fact that the epinephrine and symptomatic responses to hypoglycemia—sensitive measures of the impact of recent antecedent hypoglycemia (7,10, 11,37,38)—were not reduced significantly on day 1 provides strong evidence that recent antecedent hypoglycemia was not the cause of the reduced glucagon response.

An attenuated epinephrine response to falling plasma glucose concentrations—in the setting of an absent glucagon response—causes the clinical syndrome of defective glucose counterregulation in type 1 diabetes (1,8,9). Attenuated autonomic (including adrenomedullary epinephrine) and symptomatic responses to falling plasma glucose concentrations cause the clinical syndrome of hypoglycemia unawareness in type 1 diabetes (1). These are the central features of the concept of hypoglycemia-associated autonomic failure in type 1 diabetes (1,7,10,11). The autonomic—adrenomedullary (epinephrine), sympathetic neural (norepinephrine), and neurogenic (autonomic) symptom—responses to hypoglycemia are not eliminated; rather, the glycemic thresholds for these responses are shifted to lower plasma glucose concentrations. In other

TABLE 7
Mean (±SE) neurogenic (autonomic) symptom scores during hyperinsulinemic stepped hypoglycemic clamps on the morning and, after an additional 2 h of hypoglycemia (50 mg/dl), the afternoon of day 1 and on the morning of day 2 in nondiabetic subjects (n = 15) and patients with type 2 diabetes treated with OHA (n = 7) or insulin (n = 6)

	Time (min)												
	−30	−15	0	30	60	90	120	150	180	210	240	270	300	P
Nondiabetic subjects														
Day 1	2.3 ± 0.6	2.2 ± 0.5	2.7 ± 0.6	2.6 ± 0.5	3.2 ± 0.6	3.0 ± 0.6	3.3 ± 0.7	3.7 ± 0.7	4.0 ± 0.7	4.9 ± 0.9	6.6 ± 1.0	9.5 ± 1.7	13.1 ± 2.0	
Day 2	1.7 ± 0.5	1.5 ± 0.5	2.1 ± 0.6	2.2 ± 0.6	2.5 ± 0.6	2.6 ± 0.6	2.8 ± 0.6	3.7 ± 0.8	3.5 ± 0.6	4.7 ± 0.9	6.3 ± 4.1	7.3 ± 1.2	10.5 ± 1.4	0.1488
Type 2 diabetic patients														
OHA Rx*†														
Day 1	2.3 ± 0.2	1.6 ± 0.6	2.3 ± 0.9	2.7 ± 1.1	2.1 ± 0.8	2.9 ± 1.3	3.5 ± 1.5	7.9 ± 4.1	11.0 ± 3.	14.1 ± 4.	17.6 ± 5.	18.7 ± 4.2	20.5 ± 5.0	
Day 2	1.7 ± 1.0	1.7 ± 1.0	1.8 ± 1.0	2.0 ± 0.9	2.5 ± 0.9	3.0 ± 0.9	2.7 ± 1.1	3.3 ± 0.9	4.5 ± 0.9	4.5 ± 1.2	8.5 ± 1.7	12.8 ± 3.2	16.0 ± 4.3	0.0306
Insulin Rx*														
Day 1	2.0 ± 2.0	2.5 ± 0.9	2.8 ± 1.0	3.0 ± 0.9	2.7 ± 0.7	2.7 ± 0.8	2.8 ± 0.8	3.8 ± 0.6	4.0 ± 0.8	5.3 ± 1.3	9.0 ± 2.0	10.5 ± 2.5	21.0 ± 3.4	
Day 2	1.0 ± 1.0	1.0 ± 1.0	0.6 ± 0.6	0.6 ± 0.6	1.4 ± 1.0	1.8 ± 1.0	1.8 ± 1.0	2.2 ± 1.0	2.6 ± 1.1	3.0 ± 1.3	7.4 ± 2.2	10.8 ± 4.9	13.0 ± 4.9	0.0202

*Type 2 diabetic (n = 13) P = 0.0149 day 2 versus day 1; †day 1 P = 0.0023 versus nondiabetic.

TABLE 8
Mean (±SE) neuroglycopenic symptom scores during hyperinsulinemic stepped hypoglycemic clamps on the morning and, after an additional 2 h of hypoglycemia (50 mg/dl), the afternoon of day 1 and on the morning of day 2 in nondiabetic subjects (n = 15) and patients with type 2 diabetes treated with OHA (n = 7) or insulin (n = 6)

	Time (min)												
	−30	−15	0	30	60	90	120	150	180	210	240	270	300	P
Nondiabetic subjects														
Day 1	2.5 ± 0.5	2.3 ± 0.5	2.6 ± 0.6	2.5 ± 0.7	2.7 ± 0.5	2.7 ± 0.5	3.3 ± 0.8	3.4 ± 0.8	3.4 ± 0.7	3.5 ± 0.9	5.2 ± 1.4	8.5 ± 1.7	12.4 ±	
Day 2	2.2 ± 0.5	2.2 ± 0.7	2.5 ± 0.6	2.4 ± 0.7	2.3 ± 0.7	2.5 ± 0.7	2.7 ± 0.7	2.9 ± 0.9	3.6 ± 1.0	4.3 ± 1.1	5.6 ± 1.1	6.7 ± 1.2	9.4 ± 1.2	0.0467
Type 2 diabetic patients														
OHA Rx*														
Day 1	3.3 ± 0.8	3.0 ± 0.6	2.3 ± 0.6	4.7 ± 2.0	1.0 ± 0.5	1.3 ± 0.7	2.7 ± 1.4	6.7 ± 3.2	8.0 ± 2.4	11.1 ± 3.3	17.6 ± 5.2	18.1 ± 3.6	21.7 ± 4.0	
Day 2	0.7 ± 0.5	1.0 ± 0.6	1.3 ± 0.8	1.0 ± 0.5	0.3 ± 0.2	0.8 ± 0.6	0.8 ± 0.5	1.0 ± 0.7	2.0 ± 0.9	3.7 ± 1.5	5.3 ± 1.4	8.7 ± 2.4	11.0 ± 2.9	0.0097
Insulin Rx														
Day 1	1.5 ± 0.6	1.3 ± 0.2	2.0 ± 1.5	2.8 ± 1.2	1.8 ± 0.6	2.7 ± 0.9	2.2 ± 0.8	2.5 ± 0.8	2.3 ± 0.6	2.7 ± 0.8	10.8 ± 3.3	9.5 ± 3.1	18.5 ± 4.7	
Day 2	1.3 ± 0.7	1.3 ± 0.9	0.8 ± 0.6	1.0 ± 0.6	1.0 ± 0.6	1.2 ± 0.6	1.0 ± 0.6	1.0 ± 0.6	1.2 ± 0.7	2.0 ± 0.6	4.8 ± 1.8	8.4 ± 5.7	6.6 ± 1.4	0.0082

*Type 2 diabetic (n = 13) P = 0.0015 day 2 versus day 1; **day 1 P = 0.0182 versus nondiabetic.

FIG. 6. Mean (±SE) plasma glucagon concentrations during hyperinsulinemic stepped hypoglycemic clamps on the morning of day 1 (——) and, after an additional 2 h of hypoglycemia (50 mg/dl) the afternoon of day 1, on the morning of day 2 (- - - -) in patients with type 2 diabetes treated with OHA (n = 7). P = 0.0036

words, these responses can be elicited, but lower plasma glucose concentrations are required to elicit them. These shifts in glycemic thresholds are largely the result of recent antecedent iatrogenic hypoglycemia (1,7,10,11). Because this phenomenon is demonstrable in healthy individuals (1,37,38) and in those with type 1 diabetes (1,7,10,11), it is reasonable to anticipate that it would be demonstrable in those with type 2 diabetes. The present data document that expectation. Plasma epinephrine and norepinephrine responses and neurogenic (as well as neuroglycopenic) symptom responses to an identical hypoglycemic stimulus were reduced significantly on the day after two episodes of hypoglycemia in patients with type 2 diabetes just as they are in type 1 diabetes (7,10,11). Notably, these responses were not eliminated. Rather, lower plasma glucose concentrations were required to elicit them, i.e., the glycemic thresholds for the adrenomedullary and sympathetic neural responses and for the resultant neurogenic symptom responses were shifted to lower plasma glucose concentrations in patients with type 2 diabetes just as they are in type 1 diabetes (7,10,11).

FIG. 7. Mean (±SE) plasma epinephrine concentrations during hyperinsulinemic stepped hypoglycemic clamps on the morning of day 1 (——) and, after an additional 2 h of hypoglycemia (50 mg/dl) the afternoon of day 1, on the morning of day 2 (- - - -) in patients with type 2 diabetes treated with OHA (n = 7) P = 0.0022.

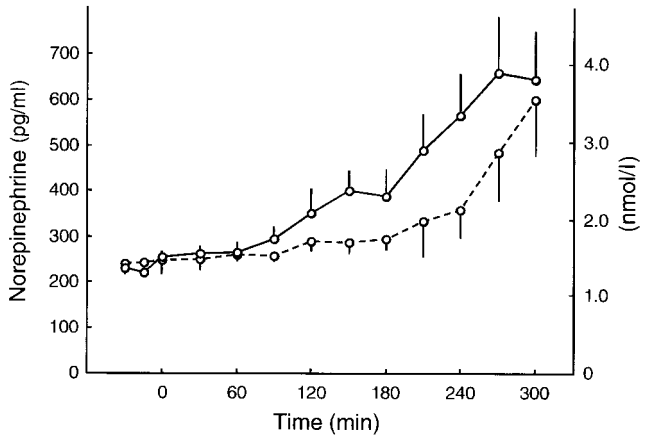

FIG. 8. Mean (±SE) plasma norepinephrine concentrations during hyperinsulinemic stepped hypoglycemic clamps on the morning of day 1 (——) and, after an additional 2 h of hypoglycemia (50 mg/dl) the afternoon of day 1, on the morning of day 2 (- - - -) in patients with type 2 diabetes treated with OHA (n = 7). P = 0.0441.

Albeit demonstrable in the patients with type 2 diabetes as a whole, the effect of recent antecedent hypoglycemia to shift the glycemic thresholds for autonomic and symptomatic responses to hypoglycemia to lower plasma glucose concentrations was most clearly demonstrated in the patients with relatively poorly controlled type 2 diabetes (mean HbA_{1c} of 8.6%) treated with OHA. In those patients, the plasma epinephrine and norepinephrine and neurogenic (and neuroglycopenic) symptom responses to hypoglycemia were enhanced compared with those of nondiabetic control subjects at baseline (day 1). These responses occurred at higher-than-normal plasma glucose concentrations. Spyer et al. (39) reported a similar pattern. Thus, glycemic thresholds for these responses are shifted to higher plasma glucose concentrations in patients with poorly controlled type 2 diabetes, just as they are in poorly controlled type 1 diabetes (40,41).

We did not study glucose recovery from hypoglycemia in type 2 diabetes. However, because the contribution of absent glucagon and attenuated epinephrine responses to falling plasma glucose concentrations has been shown in prospective studies to increase the risk of severe iatrogenic hypoglycemia 25-fold or more during aggressive therapy of type 1 diabetes (8,9), it is reasonable to suggest that patients with advanced type 2 diabetes, shown here to exhibit this pattern of counterregulatory abnormalities, are also at increased risk. If so, one could question the extent to which the higher frequency of iatrogenic hypoglycemia in insulin-treated type 2 diabetes (20,21) is the result of the greater glucose-lowering potency of insulin (given in sufficient doses) or of the impact of compromised glucose counterregulation in advanced type 2 diabetes demonstrated in the present data or both.

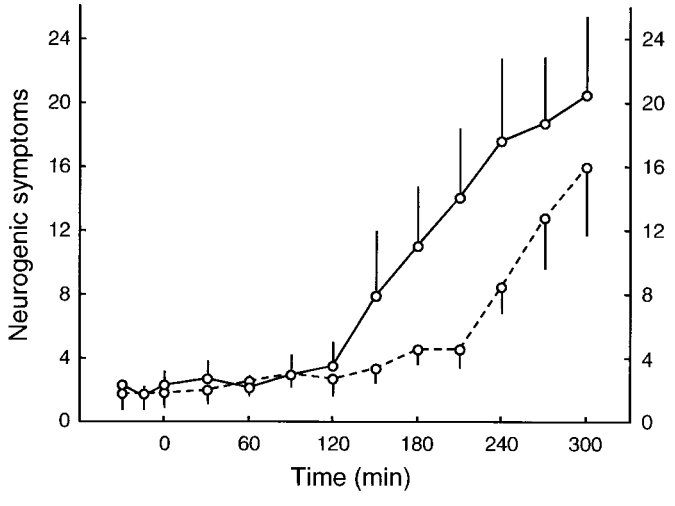

FIG. 9. Mean (±SE) neurogenic (autonomic) symptom scores during hyperinsulinemic stepped hypoglycemic clamps on the morning of day 1 (——) and, after an additional 2 h of hypoglycemia (50 mg/dl) the afternoon of day 1, on the morning of day 2 (- - - -) in patients with type 2 diabetes treated with OHA (n = 7). P = 0306.

Because the glucagon response to falling plasma glucose concentrations is virtually absent and the glycemic thresholds for autonomic (including adrenomedullary epinephrine) and symptomatic responses to hypoglycemia are shifted to lower plasma glucose concentrations by recent antecedent hypoglycemia, patients with advanced type 2 diabetes, like those with type 1 diabetes, are at risk for defective glucose counterregulation and hypoglycemia unawareness, the components of hypoglycemia-associated autonomic failure and the resultant vicious cycle of recurrent iatrogenic hypoglycemia. This may explain why iatrogenic hypoglycemia becomes limiting to glycemic control as patients approach the insulin-deficient end of the spectrum of type 2 diabetes.

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